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Palmitoylation of the adiponectin receptors, AdipoR1 and AdipoR2, is essential for function in vitro and in vivo

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Dysregulation of the adiponectin axis contributes to obesity-related cardiometabolic disorders making it an attractive therapeutic target. However, our understanding of the adiponectin receptors, AdipoR1 and AdipoR2, atypical seven transmembrane domain proteins, is rudimentary. We reasoned that elaboration of key properties of AdipoR1 and AdipoR2 would reveal therapeutic strategies. To address this we have employed *in silico*, molecular and cellular approaches.

First, using a series of complementary qualitative (microscopy) and quantitative (flow cytometry) assays we demonstrated that under steady-state conditions (no serum starvation) AdipoR1 exhibits robust (60%) cell-surface expression (CSE), whereas AdipoR2 is predominantly restricted to the ER [1]. Second, overexpression of AdipoR1 in HEK-293 cells resulted in acute activation of downstream signalling networks (AMPK, AKT, ERK and P38MAPK) whereas overexpression of AdipoR2 promoted more chronic activation (peaking at 15 min and 24 h) [2]. Third, characterisation of chimeric receptors (comprised of a series of AdipoR1/R2 and AdipoR2/R1 constructs) demonstrated that the differences in CSE and temporal signalling profiles of AdipoR1 and AdipoR2 are underpinned by the non-conserved regions (spanning $AdipoR1_{(1-70)}$ and $AdipoR2_{(1-81)}$) in the cytoplasmic 'trunks' of the receptors. Fourth, bioinformatics analysis (using CSS-Palm) revealed several putative palmitoylation sites including a conserved 'canonical' site (common to GPCRs) in the juxtamembrane region of both receptors as well as additional non-conserved sites. Palmitoylation of these sites was confirmed using Acyl-Biotinyl exchange chemistry and site-directed mutagenesis which also revealed rapid turnover of palmitoylation ($t_{1/2} < 60$ min). Moreover, palmitoylation of the canonical site in $AdipoR1_{(Cys124)}$ or $AdipoR2_{(Cys135)}$ was required for efficient CSE and coupling to downstream signalling networks (all $p < 0.05$).

Collectively these findings demonstrate fundamental differences between AdipoR1 and AdipoR2, highlight the importance of the cytoplasmic 'trunks' and post-translational regulation (palmitoylation) of the receptors. Studies are ongoing to elaborate whether changes in the latter contribute to the pathophysiology of cardiometabolic disease and afford novel therapeutic opportunities.

References

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The independent effects of dietary energy restriction and circuit exercise training on fat oxidation in patients with NAFLD

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